

Utilization of High Resolution LC-MS for Screening and Quantitative Analysis of Pesticides in Food Matrix using a Q Exactive Bench Top Orbitrap Platform

Charles Yang, Dipankar Ghosh, Jia Wang, Kristi Akervik

Thermo Fisher Scientific, San Jose, CA, USA



Introduction

The demand for quick and simple analysis of large numbers of samples in agriculture analysis is growing year by year. Throughout the world pesticides are used to control pests that are harmful to crops, humans and animals. These substances can pose a significant health threat and therefore, need to be accurately detected at the lowest levels requested by the governmental authorities typically at low part per billion (ppb) or low part per trillion (ppt) levels. Traditionally, triple quadrupoles has been used by the food industries for the identification and quantitation of these residues. The introduction of the Q Exactive provides high resolution accurate mass to unequivocally identify compounds without time consuming ms/ms optimization. The results of this unique solution are improved sensitivity and precision, as well as unmatched throughput. Mass spectrometric detection with HRAM technology using full scan experiments or Full scan data dependent ms/ms with a targeted list can deliver the ability to detect as many analytes as necessary in combination with screening for an unlimited number of compounds in a targeted list as well as untargeted approach, using only one chromatographic run. A bench top Q Exactive with the proven power of the Orbitrap mass analyzer and a novel software application for unified quantitative, confirmation and screening data processing fulfills these demands with higher confidence and precision.

Methods

Sample Preparation

Green Bell Peppers were prepared for analysis by using a modified QuEChERS (Quick, Easy, Cheap, Effective, Rugged, and Safe) method, which is a sample preparation procedure used to extract pesticides from food. The QuEChERS extracts were obtained from California Department of Food and Agriculture (CDFA). For the QuEChERS extraction, 15 g of homogenized sample and 15 mL of acetonitrile were used. Then, 200 μ L of final QuEChERS extract, 300 μ L of acetonitrile, and 500 μ L of water were transferred into an autosampler vial, spiked with 20 μ L of the pesticides standard, and mixed well. A mixture of 60 pesticides with different starting concentration was also provided by CDFA to make the standard calibration curve in neat matrix plus spiking calibration in bell pepper matrix to determine if there are ion suppression.

Liquid Chromatography

Chromatographic analysis was performed using the Thermo Scientific Accela 1250 U-HPLC system. The autosampler was an Open Accela EQuan Max HTC-Pal Autosampler (CTC Analytics, Zwingen, Switzerland). The chromatographic conditions were as follows: Column: Thermo Scientific Hypersil GOLD aQ C18 column (100 \times 2.1 mm, 1.9 μ m particle size)

Mobile Phase A: Water with 0.1% formic acid and 10 mM ammonium formate
Mobile Phase B: Methanol with 0.1% formic acid and 10 mM ammonium formate
Flow Rate: 300 μ L/min
Column Temperature: 40 $^{\circ}$ C
Sample Injection Volume: 5 μ L

Gradient:

- Time 0.00 min 98%A, 2%B
- Time 0.25 min 70%A, 30%B
- Time 10.0 min 0%A, 100%B
- Time 12.5 min 0%A, 100%B
- Time 13.0 min 98%A, 2%B
- Time 18.0 min 98%A, 2%B

Mass Spec Conditions

Full MS Scan (Targeted List)
Mass Range -120 to 1000
Positive ion mode
High Resolution: 75,000
Heated ElectroSpray Ion Source
Spray Voltage 3800V
Capillary Temp 295 $^{\circ}$ C
Sheath Gas: 32
Aux Gas: 7
Vap. Temp 295 $^{\circ}$ C

Full Scan MS Data Dependent MS/MS (Targeted List)
Mass Range - 120 to 1000
Positive ion mode
Resolution: 35,000 (Data Dependent), 75,000 (Full Scan)
Heated ElectroSpray Ion Source

Optimization of mass transitions and collision energies for each compound was not performed as full ms scan was used on a high resolution accurate mass spectrometer. Table 1 shows the list of targeted 60 pesticides analyzed.

Results

Data processing was carried out with Thermo Scientific ExactFinder software for Quantitation, Confirmation and Screening workflows. Specificity of analysis was achieved by applying a mass window of 5ppm to the theoretical mass of the analytes. All analytes gave very good linear response in the calibration range (0.01 to 0.1 ng/mL depending on starting concentration in mixture) and didn't show any interference with other analytes or matrix components (see Fig. 1, 2 and 3), the quantification data showed good reproducibility and good recovery rates.

FIGURE 1. ExactFinder displaying Boscalid Calibration Curve Plot of Matrix vs Neat, R², List of Compounds, Chromatogram and Delta ppm

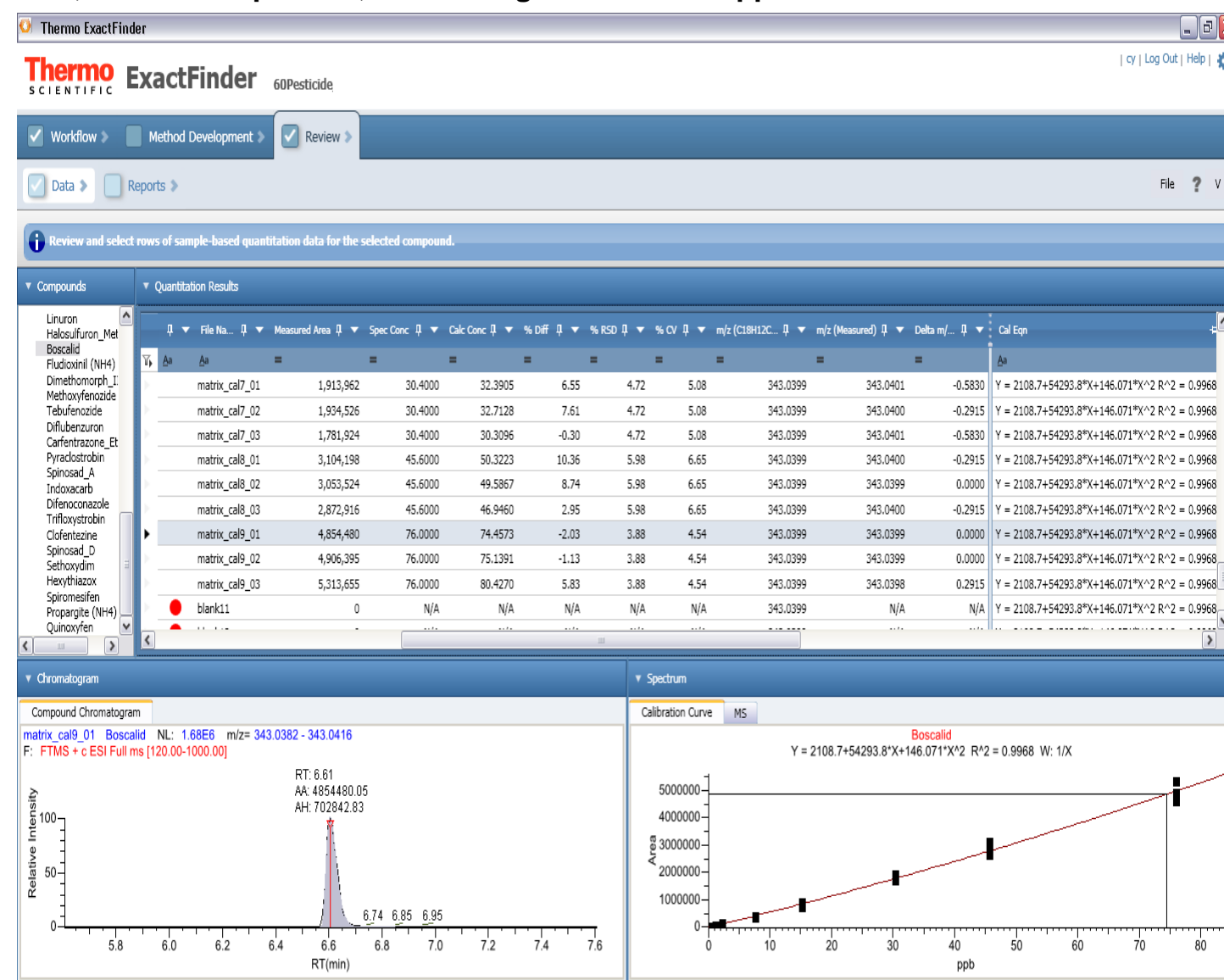


FIGURE 2. ExactFinder displaying Diuron Calibration Curve Plot of Matrix vs Neat, R², List of Compounds, Chromatogram and Delta ppm

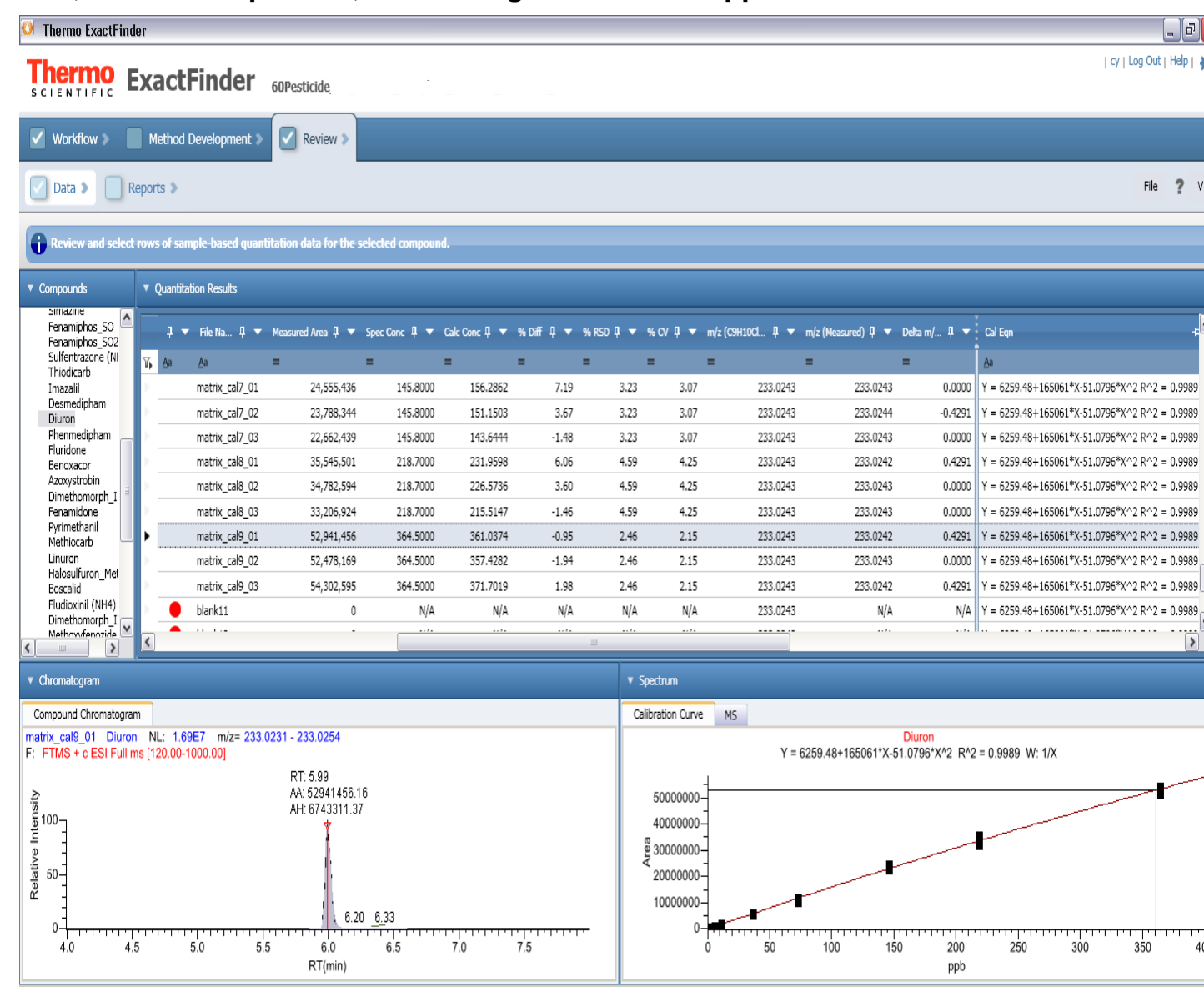
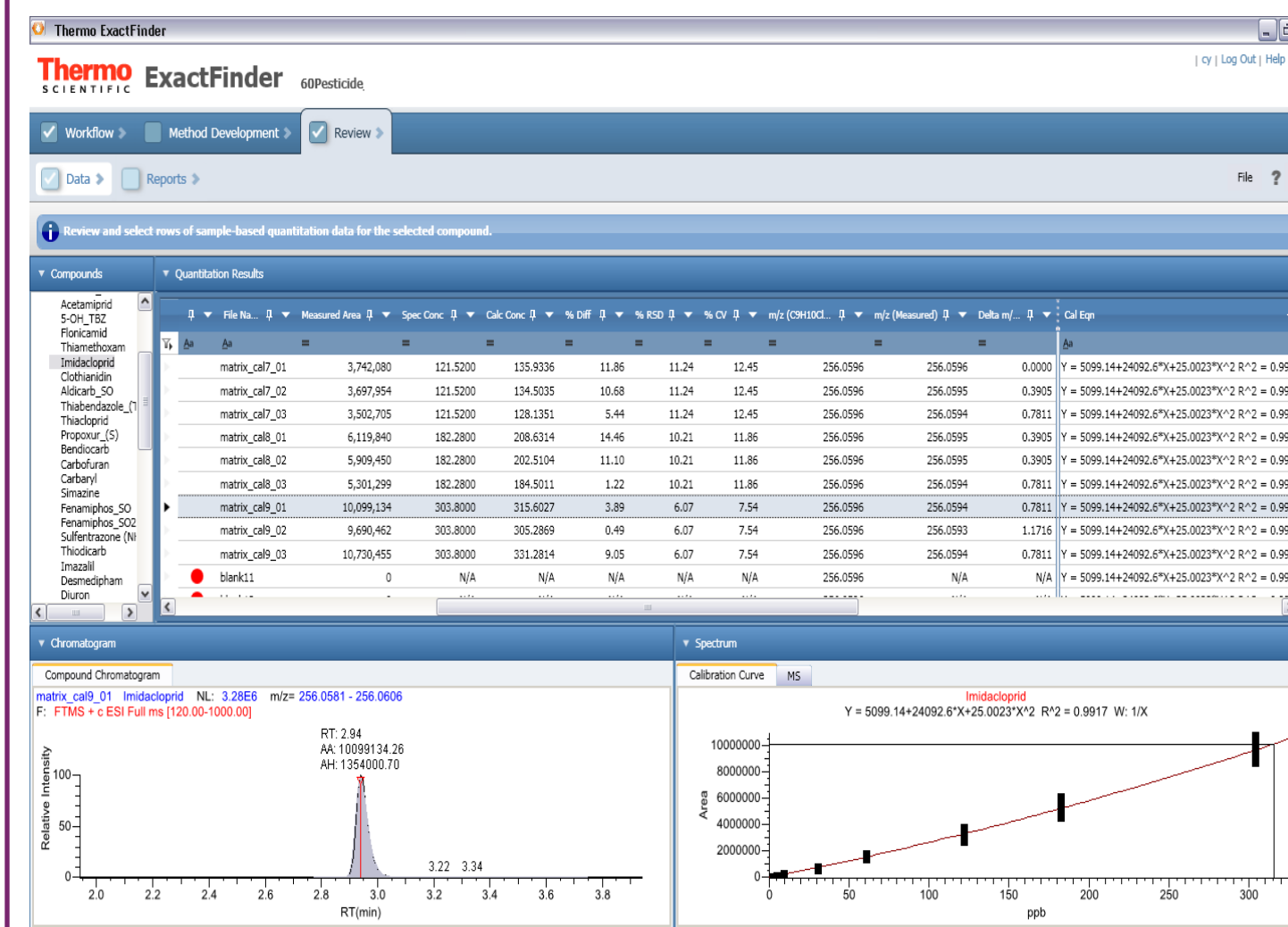


FIGURE 3. ExactFinder displaying Imidacloprid Calibration Curve Plot of Matrix vs Neat, R², List of Compounds, Chromatogram, Delta ppm



In addition to the quantitation a targeted and unknown screening was applied, using exact mass and retention time as identification criteria in the targeted screen. Confirmation of identity was achieved by automated matching of the given elemental composition with the isotopic pattern of the determined signal. Additional criteria, unused here, are occurrence of up to 5 fragment ions, library spectra match and internet database search via Chemspider. (Figure 4 and 5)

The remaining signals automatically occurred as unknowns, which again were screened against a larger compound list as unknown screen. For all signals elemental compositions were calculated on base of the isotopic distribution after defining a list of elements to be used for this calculation..

FIGURE 4. Targeted Screening hit of Difenoconazole with MS/MS Spectra Library Matching (see lower right, top spectra theoretical, lower ms/ms actual)

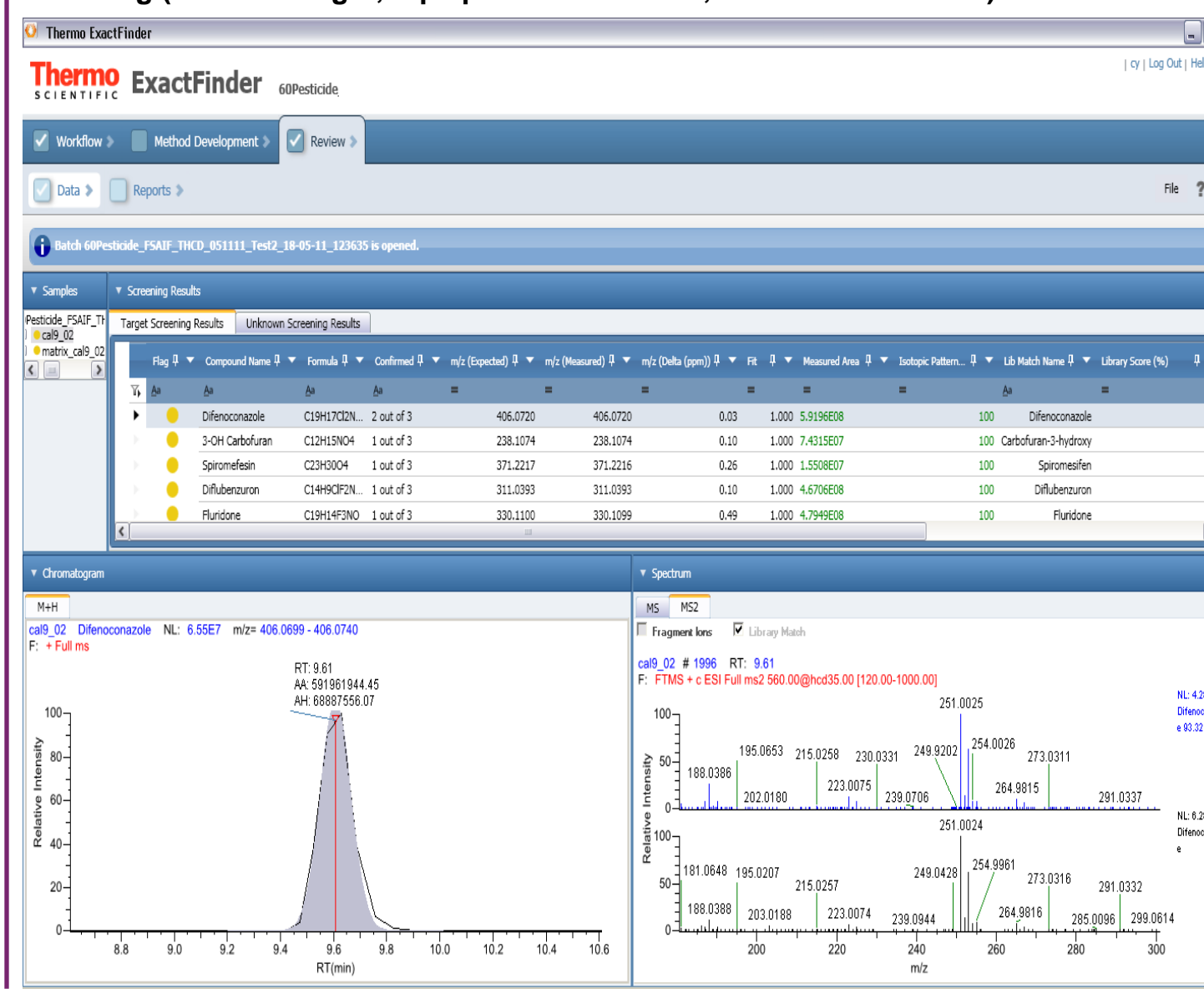
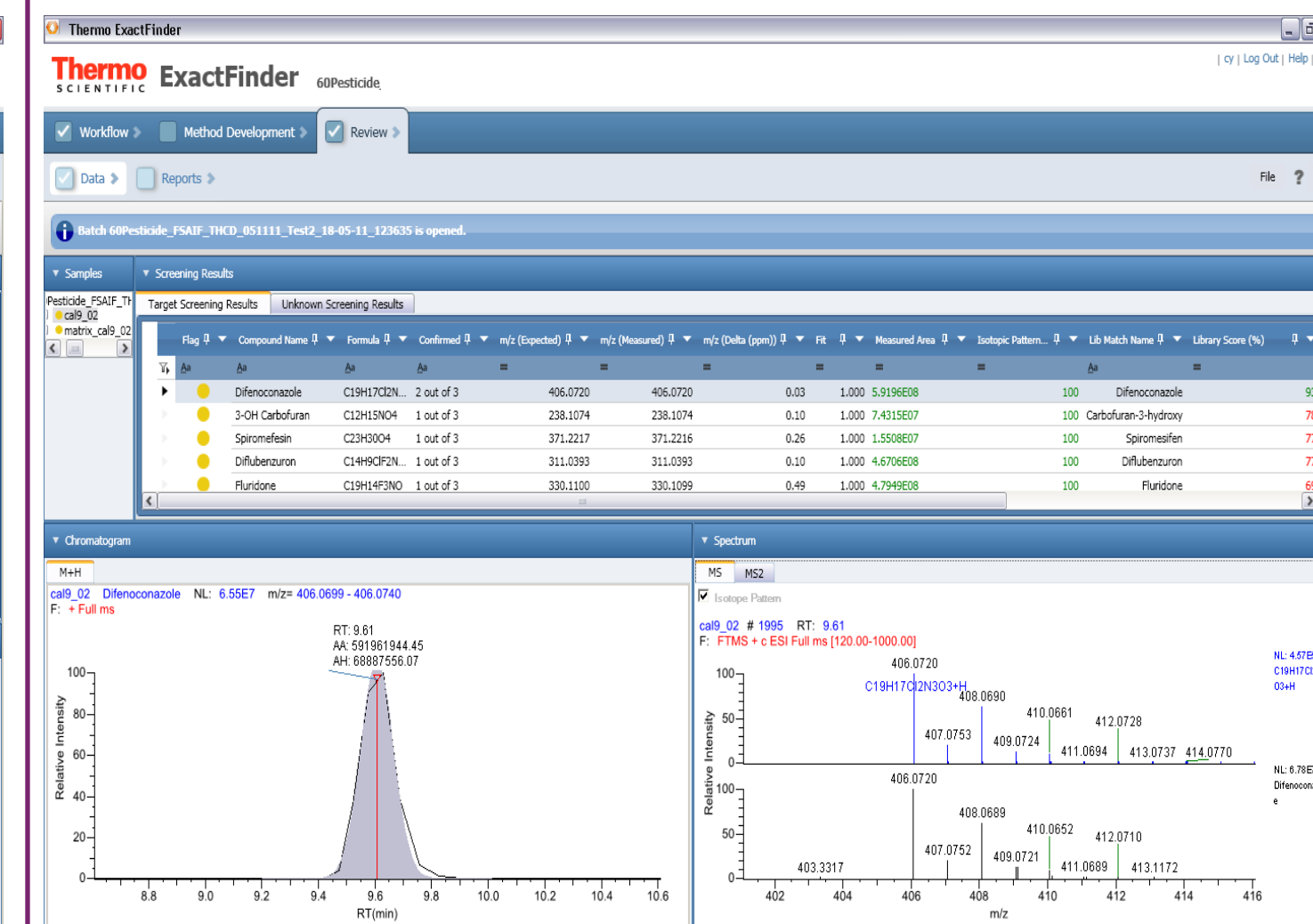


FIGURE 4. Targeted Screening hit of Difenoconazole Isotopic Pattern Matching (shown lower right, top trace theoretical, lower is actual)



The untargeted screen yielded additional compounds present in the samples. For most of the signals elemental compositions could be determined (see Fig.4). All 60 analytes of interest could be easily quantified and were assigned as knowns in the automated screen. The unknown screen yielded additional identifications of analytes without additional analytical effort. Elemental compositions were assigned to most of the so far unknown signals, leading the path into a versatile and easy to do general unknown screening (Fig. 5).

FIGURE 5. Unknown Screening hit of Chlorpyrifos oxon determined by Isotopic Pattern match using a larger pesticide database.

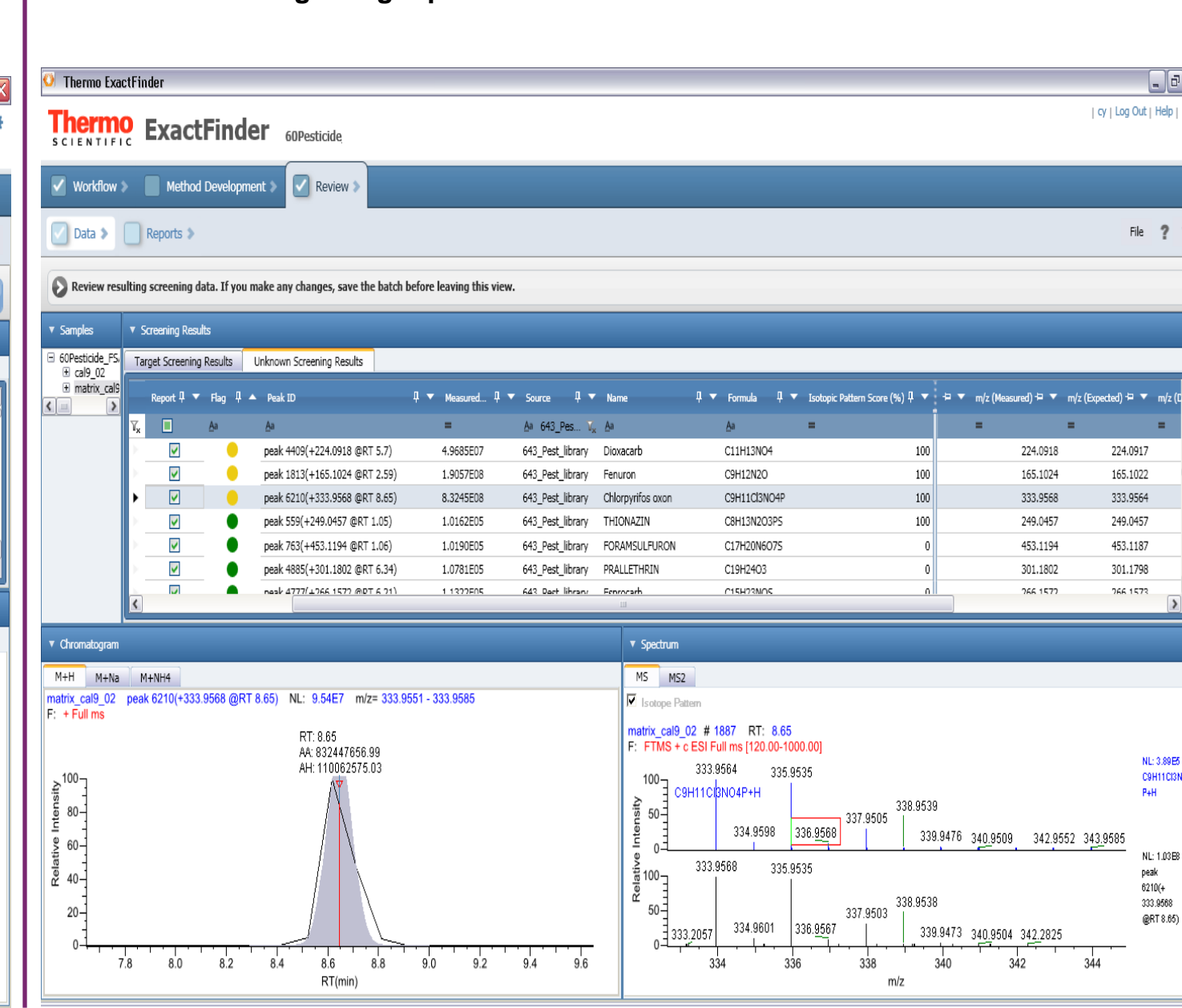


Table 1. List of 60 pesticides and it associated retention times.

Retention Time [min]	Mass [m/z]	Polarity	Start [min]	End [min]	nCE	CS	Comment
255.13390	4.85	Positive	4.85	6.85	1	3-OH Carbofuran	
218.03830	2.41	Positive	2.41	4.41	1	5-OH TBZ	
223.07452	3.32	Positive	3.32	5.32	1	Acetamidprid	
208.11140	2.23	Positive	2.23	4.23	1	Aldicarb	
223.07473	3.32	Positive	3.32	5.32	1	Aldicarb SO	
207.07981	1.78	Positive	1.78	3.78	1	Aldicarb SO2	
404.12412	6.72	Positive	6.72	8.72	1	Azoxystrobin	
224.09176	4.85	Positive	4.85	6.85	1	Bendiocarb	
260.02398	6.48	Positive	6.48	8.48	1	Boscalid	
343.03997	7.03	Positive	7.03	9.03	1	Boscalid	
202.08628	4.93	Positive	4.93	6.93	1	Carbaryl	
222.11249	4.85	Positive	4.85	6.85	1	Carbofuran	
429.07023	7.96	Positive	7.96	9.96	1	Carfentrazone Et	
303.01990	8.87	Positive	8.87	10.87	1	Clofentazid	
250.01602	2.93	Positive	2.93	4.93	1	Clothianidin	
301.11776	6.30	Positive	6.30	8.30	1	Desmedipham	
406.07200	8.83	Positive	8.83	10.83	1	Difenoconazole	
311.03936	7.98	Positive	7.98	9.98	1	Diflubenzuron	
388.13104	6.90	Positive	6.90	8.90	1	Dimethomorph I	
388.13104	7.22	Positive	7.22	9.22	1	Dimethomorph II	
203.11389	1.81	Positive	1.81	3.81	1	Dinotefuran	
233.02432	6.35	Positive	6.35	8.35	1	Diuron	
392.16046	8.33	Positive	8.33	10.33	1	Famoxadone	
312.11653	6.81	Positive	6.81	8.81	1	Fenamidone	
320.10802	5.00	Positive	5.00	7.00	1	Fenamiphos SO	
336.10293	5.18	Positive	5.18	7.18	1	Fenamiphos SO2	
230.05360	2.25	Positive	2.25	4.25	1	Fonicamid	
266.07353	7.15	Positive	7.15	9.15	1	Fludioxonil	
330.11005	6.60	Positive	6.60	8.60	1	Fluridone	
222.12373	1.65	Positive	1.65	3.65	1	Formetanate	
435.04845	7.07	Positive	7.07	9.07	1	Halosulfuron Methyl	
353.10798	9.59	Positive	9.59	11.59	1	Hexythiazox	
297.05452	6.13	Positive	6.13	8.13	1	Imazalil	
256.05960	2.86	Positive	2.86	4.86	1	Imidacloprid	
528.07801	8.74	Positive	8.74	10.74	1	Indoxacarb	
249.01923	6.97	Positive	6.97	8.97	1	Linuron	
226.08910	6.90	Positive	6.90	8.90	1	Methiocarb	
163.05360	1.68	Positive	1.68	3.68	1	Methomyl	
369.21729	7.21	Positive	7.21	9.21	1	Methoxyfenozide	
237.10156	1.97	Positive	1.97	3.97	1	Oxamyl	
163.05360	1.68	Positive	1.68	3.68	1	Oxamyl Oxime	
301.11776	6.47	Positive	6.47	8.47	1	Phermedipham	
368.18898	9.71	Positive	9.71	11.71	1	Propargite	
210.11249	4.76	Positive	4.76	6.76	1	Propoxur [S]	
200.11825	6.92	Positive	6.92	8.92	1	Pyrimethanil	
308.00400	9.87	Positive	9.87	11.87	1	Quinoxifen	
328.19357	9.12	Positive	9.12	11.12	1	Sethoxydim	
202.08542	4.93	Positive	4.93	6.93	1	Simazine	
732.46815	6.67	Positive	6.67	8.67	1	Spinosad A	
746.48380	9.06	Positive	9.06	11.06	1	Spinosad D	
371.22116	9.67	Positive	9.67	11.67	1	Spiromefesin	
404.01567	5.00	Positive	5.00	7.00	1	Sulfentrazone	
202.04337	3.50	Positive	3.50	5.50	1	TBZ	
353.22238	7.89	Positive	7.89	9.89	1	Tebufenozide	
253.03094	3.81	Positive	3.81	5.81	1	Thiacloprid	
292.02659	2.34	Positive	2.34	4.34	1	Thiamethoxam	
409.13699	8.75	Positive	8.75	10.75	1	Trifloxystrobin	

Conclusion

ExactFinder software coupled with the bench top Q Exactive provided easy access to full quantitative, confirmation and screening data in one package. The unknown search provided by the software led to the identification of a number of untargeted compounds to which in most cases elemental compositions could be assigned or by searching a larger compound database. In this case the finding of Chlorpyrifos Oxon in the unknown matrix samples shows the need to quickly move to newer technologies to help determine what we are not seeing by triple quadrupole systems.

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